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## 9 INTERPRETATION OF THE POWERPLEX® 16 BIO SYSTEM PCR AMPLIFICATION RESULTS

### 9.1 TECHNICAL NOTES

9.1.1 STR alleles are small in size, generally less than 500 bp and contain repeat units ranging from 3 to 7 bases.

9.1.2 If an allele contains an incomplete repeat, the allele is considered a microvariant and is designated by the number of complete repeats present followed by a decimal point, followed by the number of bases of the incomplete repeat. For example, the FGA 22.2 allele contains 22 tetrameric repeats plus 2 bases. Because of a deletion of two bases the FGA 22.2 allele is two bases shorter than the FGA 23 allele.

9.1.3 The characteristics of the PowerPlex® 16 BIO System and the allelic ladders are given in the table below<sup>1</sup>:

Locus	* Repeat Sequence 5' 3'	Chromosome Location	Size Range of Allelic Ladder (bp)	Alleles present in Allelic Ladder	Fluorescent Label
FGA	TTTC Complex	4q28	322-444	16-30, 31.2, 43.2, 44.2, 45.2, 46.2	Rhodamine Red™-X
TPOX	AATG	2p23-2pter	262-290	6-13	Rhodamine Red™-X
D8S1179	TCTA Complex	8q	203-247	7-18	Rhodamine Red™-X
vWA	TCTA Complex	12p12-pter	123-171	10-22	Rhodamine Red™-X
Amelogenin	NA	Xp22.1-22.3 and Y	106 – X 112 – Y	X, Y	Rhodamine Red™-X
Penta E	AAAGA	15q	379-474	5-24	Fluorescein
D18S51	AGAA	18q21.3	290-366	8-10, 10.2, 11-13, 13.2, 14-27	Fluorescein
D21S11	TCTA Complex	21q11-21q21	203-259	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38	Fluorescein
TH01	AATG	11p15.5	156-195	4-9, 9.3, 10-11, 13.3	Fluorescein
D3S1358	TCTA Complex	3p	115-147	12-20	Fluorescein

NOTE: table continued below

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Locus	*Repeat Sequence 5' 3'	Chromosome Location	Size Range of Allelic Ladder (bp)	Alleles present in Allelic Ladder	Fluorescent Label
Penta D	AAAGA	21q	376-449	2,2, 3,2, 5, 7-17	JOE
CSF1PO	AGAT	5q33.3-34	321-357	6 – 15	JOE
D16S539	AGAT	16q24-qter	264 – 304	5, 8 –15	JOE
D7S820	AGAT	7q11.21-22	215 –247	6 – 14	JOE
D13S317	AGAT	13q22-q31	169 –201	7 – 15	JOE
D5S818	AGAT	5q23.3-32	119 -155	7 – 16	JOE

\* All repeat sequences are defined using the recommendation of the DNA Commission of the International Society of Forensic Haemogenetics (ISFH): 1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of the repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used.<sup>2, 3</sup>

#### FMBIO II and FMBIO III Plus Fluorescent Image Analysis Systems:

Fluorescein is detected at a wavelength of 505 nm (FMBIO II) or 520 nm (FMBIO III Plus) - Green

Rhodamine Red<sup>TM</sup> –X is detected at a wavelength of 598 nm - Red

JOE = 6-carboxy-4',5'-dichloro 2',7' – dimethoxyfluorescein is detected at a wavelength of 577 nm - Yellow

- 9.1.4 The Fluorescent Internal Lane Standard 600 BIO (Texas Red®-X) consists of 21 DNA fragments (80,100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 bp) and can be detected at a wavelength of 665 nm (FMBIO II) or 650 nm (FMBIO III Plus) – Blue.<sup>1</sup>

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9.1.5 The “Known” Genotypes for the Control DNA (GM9947A Cell Line) using the PowerPlex® 16 BIO System are given in the table below<sup>1</sup>

Locus	Genotype GM9947A
Penta E	12,13
D18S51	15,19
D21S11	30,30
TH01	8,9.3
D3S1358	14,15
FGA	23,24
TPOX	8,8
D8S1179	13,13
VWA	17,18
Amelogenin	X,X
Penta D	12,12
CSF1PO	10,12
D16S539	11,12
D7S820	10,11
D13S317	11,11
D5S818	11,11

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<p>9.2 PROCEDURE</p> <p>9.2.1 The gel image is visually inspected to determine if the number, position, and intensity of the alleles for the allelic ladder, controls and samples are suitable for interpretation.</p> <p>9.2.1.1 If the overall quality of the gel image is unsuitable for interpretation, no further comparisons are conducted.</p> <p>9.2.1.2 If the overall quality of the gel image is suitable for interpretation, a visual comparison is performed.</p> <p>9.2.2 The source of a DNA sample may be from a single person or more than one person. This can be determined by examination of the number of alleles at each locus, optical densities and/or band intensities.</p> <p>9.2.2.1 A DNA profile may be considered to have originated from a single individual if the expected number of alleles (i.e., 1 or 2) is observed at each locus and the intensity of the alleles within a locus is approximately the same. All loci should be taken into account when making this determination.</p> <p>9.2.2.2 A sample may be considered to be a mixture of DNA from two or more individuals if the sample contains 3 or more bands at one or more loci and/or there is a distinct difference in signal intensity. All loci should be taken into account when making this determination.</p> <p>9.2.3 A visual comparison is performed between known samples and questioned samples.</p> <p>9.2.3.1 If the banding patterns of samples under comparison are distinctly different in position, it is concluded that the samples originated from different sources than the individual of interest and the individual is excluded.</p> <p>9.2.3.2 If the banding patterns of samples under comparison appear visually consistent in position, the possibility that both samples may have originated from the same source cannot be eliminated. Therefore the individual of interest is included as a possible source.</p> <p>9.2.3.2.1 Generally statistical frequencies will be generated for DNA profiles (mixture and non-mixture profiles) obtained from evidence of a probative value where the individual(s) of interest cannot be eliminated and the known standard of the individual(s) of interest contains a 1 or 2 banded patterns at each locus.</p>	

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<p>9.2.3.2.2 Statistical frequencies will not be generated at a locus if the known standard from the individual of interest exhibits a 3 or 4 banded pattern.</p> <p>9.2.3.3 If evidence samples under comparison contain a partial profile (i.e., allele dropout) or a incomplete profile (i.e., locus dropout) due to degradation, inhibition or limited DNA, the DNA profile may or may not be interpretable.</p> <p>9.2.3.3.1 All loci will be taken into account when making this determination using knowledge of the system and experience.</p> <p>9.2.3.3.2 If allele/locus dropout is observed at a majority of the loci, in order to include an individual, at least four callable loci from the evidence must match the known standard (this includes loci where masking has occurred). Otherwise the partial profile will be reported as inconclusive or may be used only for elimination purposes. However, additional information such as the presence of a rare allele observed only in a small portion of the population will be taken into consideration in consultation with a supervisor and/or the Section Chief when reaching a conclusion.</p> <p>9.2.3.4 When a probative result is obtained for an evidence sample and an inconclusive result is obtained at more than one locus for a known reference sample, the known reference sample will be re-typed or re-amplified in order to produce a complete DNA profile.</p> <p>9.2.3.5 In criminal paternity/maternity and missing person cases, an individual must be eliminated at three or more loci to account for the possibility of mutations before the individual is eliminated as a parent/offspring.</p> <p>9.2.3.5.1 When a couple is evaluated as possible biological parents of a missing person, each possible parent's DNA profile will be evaluated separately to determine if the individual is included or eliminated as a biological parent. Subsequently the profiles from both individuals will be evaluated together to determine if as a couple they could have conceived the missing person.</p> <p>9.2.4 All casework samples (i.e., evidence samples, known standards) and positive amplification controls must be adjacent to an allelic ladder and an internal lane standard should be used to determine the allele designation. It is not necessary for the reagent blanks, the negative amplification control, or plate blanks to be loaded next to an allelic ladder.</p> <p>9.2.5 Following a visual examination of the gel image, if the samples appear visually consistent, the amplified alleles are designated by noting which allelic ladder band(s) lines up with the sample band(s), as demonstrated in the examples in section 9.3.</p>	

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<p>9.2.6 Allele designations will be determined for each sample by the examiner/analyst using the FMBIO Fluorescent Imaging System STaRCall software. Refer to Appendix F (FMBIO II) or Appendix O (FMBIO III Plus) for the FMBIO Fluorescent Imaging Analysis System procedure and instructions on assigning allele designations using the STaRCall software.</p> <p>9.2.7 An independent calling of the alleles for each sample will be conducted by a second qualified individual using the FMBIO Fluorescent Imaging Analysis System.</p> <p>9.2.7.1 If the examiner/analyst and the independent sizer's allele designations are in agreement the alleles can be called.</p> <p>9.2.7.2 If the examiner/analyst and the independent sizer's allele designations are different and no agreement can be reached, the results for the allele(s) in question will be called inconclusive. If possible, the sample(s) will be re-analyzed.</p> <p>9.2.7.3 When the examiner evaluates his/her sizing data and compares the results to the independent sizer's results, if one examiner uses parentheses and the other does not, providing the alleles for the sample at the locus in question have been called the same by both examiners, the allele designation (i.e., parentheses vs. no parentheses) which provides the most conservative result will be used.</p> <p>NOTE: It is not necessary to size or have the typing gel independently sized when no probative results are obtained; visual comparisons may be used.</p> <p>9.2.8 The examiner's/analyst's allele calls are the "official" values entered into CODIS.</p> <p>9.2.9 If an allele is seen in a region "off- ladder", i.e., above the top allelic ladder band of the upper most locus on the typing gel, below the bottom allelic ladder band of the bottom most locus on the typing gel, or between loci, an allele designation based upon the nomenclature referenced in step 9.2.9.1 will be used. However, to ensure that the evidence and standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., vWA 8).</p> <p><b>EXCEPTION:</b> If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the nomenclature referenced in step 9.2.9.1. When the suspect's standard is subsequently analyzed, assuming there is an off-ladder allele, the same nomenclature referenced in step 9.2.9.1 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., vWA 8) to ensure both alleles are the same.</p>	

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<p>9.2.9.1 If the two bands are determined to be the same and the allele is seen above the top allelic ladder band of the upper most locus on the typing gel, it will be assigned the type of the top allele of the allelic ladder with a greater than sign (&gt;). If the allele is seen below the bottom allelic ladder band of the bottom most locus on the typing gel, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (&lt;). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.</p> <p>9.2.10 If an allele is seen between two loci and either the locus above <b>OR</b> below the band contains two bands the allele will be considered to belong with the locus containing the single band. The assignment of the allele designation will be based upon the nomenclature referenced in step 9.2.10.1. However, to ensure that the evidence and standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., D5S818 17).</p> <p><b>EXCEPTION:</b> If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the nomenclature referenced in step 9.2.10.1. When the suspect's standard is subsequently analyzed, assuming there is an off-ladder allele, the same nomenclature referenced in step 9.2.10.1 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., D5S818 17) to ensure both alleles are the same.</p> <p>9.2.10.1 If the two bands are determined to be the same and the allele is above the top allelic ladder band of the locus containing the single band, it will be assigned the type of the top allele of the allelic ladder with a greater than sign (&gt;). If the allele is below the bottom allelic ladder band of the locus containing the single band, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (&lt;). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.</p> <p>9.2.11 If an allele is seen between two loci and the locus above <b>AND</b> below the band contains a single band, follow the guidelines outlined in steps 9.2.11.1 through 9.2.11.3.</p> <p><b>EXCEPTION:</b> If the evidence sample is from a non-subject case, the sample will be typed and assigned an allele designation based upon the procedure outlined in steps 9.2.11.1 through 9.2.11.3. When the suspect's standard is analyzed, assuming there is an off-ladder allele, the same procedure outlined in steps 9.2.11.1 through 9.2.11.3 will be used. The base pair values for the evidence and suspect's standard samples will be compared to the lookup table associated with the analysis of the sample to assign the repeat unit value (i.e., D5S818 17) to ensure both alleles are the same.</p>	

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<div data-bbox="435 352 1549 590"> <p>9.2.11.1 The base pair size for the allele in question will be compared to the base pair values for the top allelic ladder band of the lower molecular weight locus and to the bottom allelic ladder band of the higher molecular weight locus. In addition, the optical density for the allele in question will be compared to the optical density of the single allele in higher and lower molecular weight loci, and an evaluation of the physical location of the allele in question to the higher and lower molecular weight loci will be conducted.</p> </div> <div data-bbox="435 636 1549 772"> <p>9.2.11.2 The allele in question will be considered to belong to the locus which is closest in proximity, contains an allele of approximately the same optical density and falls within an appropriate size distance from the locus (i.e., one or two repeat units from the top/bottom allelic ladder band).</p> </div> <div data-bbox="435 819 1549 1157"> <p>9.2.11.3 Once it has been determined to which locus the allele belongs, if the allele is above the top allelic ladder band it will be assigned the type of the top allele of the allelic ladder with a greater than sign (&gt;). If an allele is below the bottom allelic ladder band, the allele will be assigned the type of the bottom allele of the allelic ladder with a less than sign (&lt;). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation. To ensure that the evidence and the standard samples contain the same allele, the base pair values for the evidence sample and the standard will be compared to the lookup table to verify that both samples would be assigned the same repeat unit value (i.e., D5S818 17).</p> </div> <div data-bbox="341 1203 1549 1472"> <p>9.2.12 If an allele, in relationship to the allelic ladder, is less than 300 bp in size, is visually between two allelic ladder bands, the STaRCall program generates a value "out of range" and the base pair value is +/- 1.0 base pair or greater from that of the "Known" repeat base pair value, the allele will be considered to be a microvariant. The allele will be assigned an allele designation of the lower repeat value followed by the number of bases in the incomplete repeat. Example: an allele which migrates one base pair below the TH01 9 allele will be designated as a TH01 8.3. The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.</p> </div> <div data-bbox="435 1518 1549 1619"> <p>NOTE: The allele designation can be determined manually or using the procedure addressed in Step 5.11 of Appendix F (FMBIO II) or Appendix O (FMBIO III Plus) Fluorescent Detection of The Electrophoresis Gel.</p> </div> <div data-bbox="341 1665 1549 1902"> <p>9.2.13 If an allele/band is equal to or greater than 300 bp in size, is visually between two allelic ladder bands (i.e., the allele is not present in the allelic ladder) and/or the STaRCall program generates a value out of range and the base pair value is +/- 1.0 base pair or greater from that of the "Known" repeat base pair value, the allele/band will be considered to be a microvariant. In order to confirm the allele is a microvariant the typing gel may be placed back into the electrophoresis tank and run for a sufficiently longer period of time or the evidence sample(s) and standard may be re-loaded onto a second typing gel. The typing gel will be run for a</p> </div>	

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<p>sufficiently longer period of time to provide better separation of the allelic ladder bands, thereby attaining better precision in measurement for assigning the allele designation.</p> <p>EXCEPTION: The Penta D “6 allele”, which is not in the allelic ladder, will not be viewed as a microvariant. The assignment of the allele designation for the Penta D “6 allele” will be based upon the base pair size in relation to the base pair size of the Penta D 5 and 7 alleles.</p> <p>NOTE: If the typing gel will not be run for a sufficiently longer period of time the allele must be reported as inconclusive.</p> <p>To confirm a microvariant the typing gel must contain a positive amplification control and have been independently sized.</p> <p>9.2.13.1 Only the allele(s) for the locus in question will be sized.</p> <p>9.2.13.2 Based upon the base pair value obtained, the allele/band will be assigned an allele designation of the lower repeat value followed by the number of bases in the incomplete repeat (Example: an allele which migrates one base below the Penta E 10 allele will be designated as a Penta E 9.4). The "out of range" value on the STaRCall spreadsheet will be manually changed to reflect the allele designation.</p> <p>9.2.13.3 The allele designations generated from this typing gel will be the official allele designations.</p> <p>NOTE: The allele designation can be determined manually or using the procedure addressed in Step 5.11 of Appendix F (FMBIO II) or Appendix O (FMBIO III Plus) Fluorescent Detection of The Electrophoresis Gel.</p> <p>9.2.14 If a single band above 300 bp, based upon the band morphology/intensity, appears to be the coalescing of two closely spaced bands (i.e., one base pair apart, such as FGA 22 and 22.1), or the evidence sample and standard generated allele designations that are a single base pair different and the allele is relevant to the case, the typing gel may be placed back into the electrophoresis tank and run for a sufficiently longer period of time or the evidence sample(s) and standard may be re-loaded onto a second typing gel. The typing gel will be run for a sufficiently longer period of time, then re-scanned at the locus in question to resolve the possible coalesced band(s) or the difference between the evidence sample and standard.</p> <p>9.2.14.1 Only the allele(s) for the locus in question will be sized.</p> <p>9.2.14.2 The allele designations generated from this typing gel will be the “official” allele designationS.</p>	

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<p>NOTE: If the typing gel will not be run for a sufficiently longer period of time to resolve the possible coalesced band(s), the locus must be reported as inconclusive.</p> <p>9.2.15 If an allele falls between the FGA 31.2 and the 43.2 allelic ladder bands, the allele will be assigned an allele designation of &gt;31.2.</p> <p>9.2.16 If an allele (i.e., microvariant, off-ladder allele or rare allele) has been observed 5 times or less in the Department of Forensic Science population database, an allele frequency of 5/n will be assigned to the allele.</p> <p>9.2.17 To indicate the gender of the contributor of a particular biological sample the Amelogenin locus may be used. A biological sample exhibiting a single band at approximately 106 bp (X allele) will generally be considered to have originated from a female individual. A biological sample exhibiting a band at approximately 106 bp (X allele) and a band at approximately 112 bp (Y allele) will generally be considered to have originated from a male individual. If only a Y allele is obtained or only an X allele is obtained from a known male sample, the results at the Amelogenin locus will be reported as inconclusive. Refer to Technical Notes 9.1.3 and 9.1.5 for additional information about the Amelogenin locus.</p> <p>9.2.18 For a typing result to be reported all controls must work appropriately.</p> <p>9.2.18.1 Reagent Blanks</p> <p>9.2.18.1.1 If a weak signal is detected in a reagent blank at a single locus and the signal is demonstrated to be part of the control, the test results associated with the reagent blank will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-extracted and/or re-amplified.</p> <p>9.2.18.1.2 If a weak signal is detected in a reagent blank at multiple loci, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.</p> <p>9.2.18.1.3 If a strong signal is detected in a reagent blank at a single locus or at multiple loci, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.</p> <p>9.2.18.2 For convicted offender and arrestee sample analysis, the Random Sample profile when searched in CODIS must elicit the correct result. If the DNA</p>	

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<p>sample number identified as a result of the search is different from the DNA sample number on the master list (maintained by the Forensic Biology Section Chief or designee) all samples associated with the Random Sample will be re-extracted and/or re-amplified if possible.</p> <p>9.2.18.3 The Control DNA (GM9947A Cell Line) must elicit the "Known" genotype for each locus as specified in Technical Note 9.1.5. If an allele is detected in the Control DNA at a specific locus that is not consistent with the known genotype, the test will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.</p> <p>9.2.18.4 Negative Amplification Control</p> <p>9.2.18.4.1 If a weak signal is detected in the negative amplification control at a single locus and the signal is demonstrated to be part of the control, the test results associated with the negative amplification control will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.</p> <p>9.2.18.4.2 If a weak signal is detected in the negative amplification control at multiple loci, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.</p> <p>9.2.18.4.3 If a strong signal is detected in the negative amplification control at a single locus or at multiple loci, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.</p> <p>9.2.18.5 If no typing result is observed for the Random Sample (for convicted offender and arrestee sample analysis) or the Control DNA (GM9947A Cell Line) at a particular locus all samples at that locus will be considered inconclusive. If it is deemed necessary, such as the locus provides exculpatory information, the sample(s) associated with the Random Sample or Control DNA will be re-amplified and typed.</p> <p>9.2.19 When a band stronger in intensity is accompanied by a band weaker in intensity that has migrated one allele position (n-4) farther than the more intense band, this may be a stutter band.</p> <p>NOTE: Stutter may also be seen at an n+4 position to that of the more intense band. In addition, if the sample is overloaded, a high concentration of DNA was amplified, or the sample is degraded,</p>	

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artifactual bands may also be seen at n-1, n-2, n-3 and n-8 positions to the intense band or the stutter band may have an elevated optical density values <sup>4</sup> .																																																											
9.2.19.1		Both the strong and weak band will be sized using the STaRCaI allele calling software. Refer to Appendix F (FMBIO II) or Appendix O (FMBIO III Plus) for the FMBIO Fluorescent Imaging Analysis System procedure.																																																									
9.2.19.2		All loci must be taken into account when determining if a sample is a mixture of biological material from more than one source. In order to determine if a weak band in an n-4 position is the result of normal stutter within a locus or a mixture of two or more sources of biological material, the analyst will use experience and/or the percent stutter values to make an informed decision. If the ratio of the OD (optical density) for the strong band to the weak band is less than the established values (listed below) the band may be considered to be stutter. The following percent stutter values will serve as a guide:																																																									
<table><tr><td>Locus</td><td>% Stutter (FMBIO II)</td><td>% Stutter (FMBIO III Plus)</td><td>Locus</td><td>% Stutter (FMBIO II)</td><td>% Stutter (FMBIO III Plus)</td></tr><tr><td>FGA</td><td>11.0</td><td>13.0</td><td>D3S1358</td><td>12.0</td><td>16.0</td></tr><tr><td>TPOX</td><td>8.0</td><td>11.0</td><td>Penta D</td><td>2.0**</td><td>2.0**</td></tr><tr><td>D8S1179</td><td>10.0</td><td>12.0</td><td>CSF1PO</td><td>11.0</td><td>13.0</td></tr><tr><td>VWA</td><td>16.0</td><td>16.0</td><td>D16S539</td><td>12.0</td><td>13.0</td></tr><tr><td>Penta E</td><td>2.0**</td><td>2.0**</td><td>D7S820</td><td>11.0</td><td>12.0</td></tr><tr><td>D18S51</td><td>13.0</td><td>13.0</td><td>D13S317</td><td>10.0</td><td>11.0</td></tr><tr><td>D21S11</td><td>15.0</td><td>15.0</td><td>D5S818</td><td>13.0</td><td>13.0</td></tr><tr><td>TH01</td><td>5.0</td><td>5.0</td><td></td><td></td><td></td></tr></table>						Locus	% Stutter (FMBIO II)	% Stutter (FMBIO III Plus)	Locus	% Stutter (FMBIO II)	% Stutter (FMBIO III Plus)	FGA	11.0	13.0	D3S1358	12.0	16.0	TPOX	8.0	11.0	Penta D	2.0**	2.0**	D8S1179	10.0	12.0	CSF1PO	11.0	13.0	VWA	16.0	16.0	D16S539	12.0	13.0	Penta E	2.0**	2.0**	D7S820	11.0	12.0	D18S51	13.0	13.0	D13S317	10.0	11.0	D21S11	15.0	15.0	D5S818	13.0	13.0	TH01	5.0	5.0			
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D21S11	15.0	15.0	D5S818	13.0	13.0																																																						
TH01	5.0	5.0																																																									
** No stutter was observed during the validation studies. Therefore, the percent stutter specified is based upon recommendations of the manufacturer reported in the PowerPlex® 2.1 System Technical Manual.																																																											
9.2.19.3		If the ratio of the OD (optical density) for the strong band to the weak band is less than the established stutter value (listed above), the allele will be considered to be stutter and will not be called even if it is believed that the band is a true allele.																																																									
9.2.19.4		If the ratio of the OD (optical density) for the strong band to the weak band is above the established stutter value (this event is generally observed in samples containing a high concentration of DNA or the DNA is partially degraded),																																																									

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the allele may still be called stutter once all loci have been taken into account and both the examiner/analyst and the independent sizer are in agreement (based upon knowledge of the system and experience) that the less intense band is stutter. The examiner will type "ST" in the percentage column of the STaRCall spreadsheet to signify that what was observed was considered to be stutter.

- 9.2.20 When a strong band in intensity and a weak band in intensity are observed within a single locus and the bands are separated by greater than one repeat unit, the difference in intensity could be the result of a null allele or the inability of the primer to bind to the template fully in the flanking region of one of the alleles. In order to determine if a DNA profile containing bands with a difference in intensity is a result of a null allele, primer mis-pairing or a mixture of biological material from more than one source, the analyst must take into account all of the loci, use experience and/or the heterozygous percent intensity values to make an informed decision. If the percentage values obtained from the ratio of the OD (optical density) for the stronger band to the weaker band is equal to or greater than the established values (listed below), both bands within the locus may be considered to have originated from a single donor. The following heterozygous percent intensity values will serve as a guide:

Locus	Lower Limit Difference Between Two Heterozygous Alleles (3 STD below the mean)	Locus	Lower Limit Difference Between Two Heterozygous Alleles (3 STD below the mean)
Penta E	45%	VWA	65%
D18S51	54%	Amelogenin	N/A
D21S11	63%	Penta D	No data available at this time
TH01	70%	CSF1PO	62%
D3S1358	67%	D16S539	64%
FGA	57%	D7S820	63%
TPOX	63%	D13S317	66%
D8S1179	63%	D5S818	68%

Note: Data generated from internal validation conducted by the Virginia Department of Forensic Science

- 9.2.21 If it is determined that a sample contains stutter bands at a majority of the loci and other artifactual bands are visible throughout the sample lane due to overloading of the sample and/or amplifying too much sample DNA, the sample will be re-amplified with less template DNA and then re-typed. A review of the product gel results may be needed to ensure that overloading of the sample has not occurred.

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<div data-bbox="431 350 1494 451"> <p>9.2.21.1            If it is determined that a band of lesser intensity in relationship to a more intense band is a true allele, the allele(s) will be reported on the landscape spreadsheet inside parentheses ( ).</p> </div> <div data-bbox="339 501 1544 802"> <p>9.2.22    When comparing the bands on the gel image to the allele calls generated by the STaRCall program, if the band visually is too weak to call or it is unclear whether the area that was marked by the computer program is actually a band, the examiner will manually type in a double question mark (??) in the percentage column of the STaRCall spreadsheet to signify that something was observed; however no conclusions could be made. If an artifactual band (i.e., n-1, n-2, n-3 or n-8) is observed due to overloading of the sample, the examiner will manually type “ART” in the percentage column of the STaRCall spreadsheet. If a band is believed to have originated from another channel and therefore is a result of bleed through, the examiner will manually type “BT” in the percentage column of the STaRCall spreadsheet.</p> </div> <div data-bbox="431 852 1534 951"> <p>9.2.22.1            As a result of the out of range allele(s)/artifact(s) being listed out of sequence on the STaRCall spreadsheet, the examiner will record on the STaRCall spreadsheet to which locus the band/artifact is associated.</p> </div>	

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9.3 INTERPRETATION OF POWERPLEX® 16 BIO SYSTEM ALLELES				
Amplified PowerPlex® 16 BIO System alleles are typed by noting which allelic ladder band(s) lines up with the test sample band(s), as demonstrated in the following examples:				
	C	L	S	
46.2				FGA
45.2		—	—	
44.2		—		
43.2		—		
31.2		—		
30		—		
29		—		
28		—	—	
27		—		
26		—		
25		—		TPOX
24	—	—		
23	—	—		
22		—		
21		—		
20		—		
19		—		
18		—		
17		—		
13		—	—	D8S1179
12		—		
11		—		
10		—	—	
9		—		
8	—	—		
7		—		
6		—		
18		—		vWA
17		—	—	
16		—		
15	—	—		
14		—		
13		—		
12		—		
11		—		
10		—		
22		—	—	Amelogenin
21		—		
20		—		
19		—		
18	—	—		
17	—	—		
16		—	—	
15		—		
14		—		
13		—		
12		—		
11		—		
10		—		
Y		—	—	
X	—	—	—	

In this example, the FGA alleles for the sample lane (S) line up with alleles 28 and 45.2 of the allelic ladder (L), the TPOX alleles line up with alleles 10 and 12 of the allelic ladder, the D8S1179 allele lines up with allele 16 of the allelic ladder, the vWA alleles line up with alleles 15 and 21 of the allelic ladder, and the Amelogenin alleles line up with the X and Y alleles of the allelic ladder. Therefore, this sample has a genotype of FGA - 28, 45.2; TPOX - 10,12; D8S1179 – 16; vWA - 15,21; and Amelogenin X,Y. The Control DNA (9947A Cell Line designated as C) which is run on every gel has a genotype of FGA -23,24; TPOX – 8; D8S1179 – 13; vWA - 17,18; and Amelogenin X,X.

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INTERPRETATION OF A FOREIGN PROFILE FROM A MIXTURE SEARCHED IN CODIS (COMBINED DNA INDEX SYSTEM):					
Example:					
Channel 4 – JOE					
	V	L	E		
17		—			
16		—			
15		—			
14		—			
13	—	—	—		
12		—	....		
11		—			
10		—		Penta D	
9	—	—	—		
8		—	....		
7		—			
5		—			
3.2		—			
2.2		—			
15		—			
14	—	—	—		
13		—	....		
12		—			
11		—		CSF1PO	
10	—	—	—		
9		—			
8		—	....		
7		—			
6		—			
15		—			
14		—	....		
13		—			
12	—	—	—		
11	—	—	==	D16S539	
10		—			
9		—			
8		—			
5		—			
14		—			
13		—			
12		—	....		
11	—	—	—		
10		—	....	D7S820	
9	—	—	—		
8		—			
7		—			
6		—			
15		—			
14		—			
13		—			
12		—	....	D13S317	
11		—			
10		—			
9		—			
8	—	—	—		
7		—			
15		—			
14		—			
13		—	....	D5S818	
12	—	—	—		
11	—	—	==		
10		—			
9		—			
8		—			
7		—			
Note: .... Indicates an allele of weaker intensity == Indicates an allele of stronger intensity					

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<p>In the above example, the victim (V) has the following profile: Penta D 9, 13; CSF1PO 10, 14; D16S539 11,12; D7S820 9,11; D13S317 8; and D5S818 11,12. Therefore, the minimum known foreign DNA profile that would be searched in CODIS would be:</p> <table><tr><td>Penta D</td><td>8, 12</td><td>(weaker intensity alleles)</td></tr><tr><td>CSF1PO</td><td>8, 13</td><td>(weaker intensity alleles)</td></tr><tr><td>D16S539</td><td>11, 14</td><td>The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 14 allele is foreign to the victim.</td></tr><tr><td>D7S820</td><td>10, 12</td><td>(weaker intensity alleles)</td></tr><tr><td>D13S317</td><td>12</td><td>(weaker intensity allele)</td></tr><tr><td>D5S818</td><td>11, 13</td><td>The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 13 allele is foreign to the victim.</td></tr></table> <p><b>REFERENCES:</b></p> <ol style="list-style-type: none"><li>PowerPlex® 16 BIO System Technical Manual</li><li>Bär W. <i>et al.</i> (1997) DNA recommendations: further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems, <i>Int. J. Legal Med.</i> <b>110</b>, 175.</li><li>Gill, P. <i>et al.</i> (1997) Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. <i>Forensic Science International</i> <b>87</b>, 185-192.</li><li>Gill, P. <i>et al.</i> (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, <i>Forensic Science International</i> <b>112</b>, 17-40.</li><li>Curran, J.M., <i>et al.</i> (2005) Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure, <i>Forensic Science International</i> <b>148</b>, 47-53.</li></ol>		Penta D	8, 12	(weaker intensity alleles)	CSF1PO	8, 13	(weaker intensity alleles)	D16S539	11, 14	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 14 allele is foreign to the victim.	D7S820	10, 12	(weaker intensity alleles)	D13S317	12	(weaker intensity allele)	D5S818	11, 13	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 13 allele is foreign to the victim.
Penta D	8, 12	(weaker intensity alleles)																	
CSF1PO	8, 13	(weaker intensity alleles)																	
D16S539	11, 14	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 14 allele is foreign to the victim.																	
D7S820	10, 12	(weaker intensity alleles)																	
D13S317	12	(weaker intensity allele)																	
D5S818	11, 13	The 11 allele is twice as intense as the 12 allele which is provided by the victim. Therefore, a conclusion can be reached that the contribution of the 11 allele based on the intensity is not solely from the victim. The 13 allele is foreign to the victim.																	
◆END																			